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Notes on Reproduction of the Emerald Skink, *Lamprolepis smaragdina* (Squamata: Scincidae) from Papua New Guinea

Abstract

A histological examination was conducted on gonadal material of the scincid lizard, *Lamprolepis smaragdina* from Papua New Guinea. Males exhibited a prolonged period of sperm formation. The smallest reproductively active male measured 96 mm SVL. Mean clutch size for 10 females was 1.9 ± 0.32 SD, range: 1-2. Female *L. smaragdina* produce multiple clutches in the same year. The smallest reproductively active female measured 83 mm SVL. Examination of other populations of *L. smaragdina* are needed to determine whether geographic variation in the reproductive cycle exists.

Introduction

Lamprolepis smaragdina is widespread in Oceania and occurs in the Admiralty Islands, Marshall Islands, Indonesia, New Guinea, Solomon Islands and Taiwan (Crombie and Pregill, 1999). There is previous information on captive *L. smaragdina* (Peters, 1985; Rogner, 1977) and from wild Philippine and Papua New Guinea populations (Alcala, 1966; Auffenberg and Auffenberg, 1989; Goldberg and Kraus, 2008) as well as clutch sizes from the Solomon Islands (McCoy, 2006). The purpose of this note is to add information on the reproduction of *L. smaragdina* from Papua New Guinea as part of an ongoing series of studies in which we characterize the reproductive cycles of lizards from Oceania.

Thirty *L. smaragdina* eleven males (mean snout-vent length, SVL = $102.2 \text{ mm} \pm 3.4$ SD, range = 96-106 mm), thirteen females (mean SVL = $95.8 \text{ mm} \pm 8.2$ SD, range = 81-105 mm) and six juveniles (mean SVL = $61.3 \text{ mm} \pm 20.1$ SD, range = 33-77 mm) collected by CCA from Papua New Guinea were borrowed from the herpetology collection (LSUMZ) of the Museum of Natural Science, Louisiana State University, Baton Rouge, Louisiana, USA and examined for helminths. Twenty were from Sandaun Province (LSUMZ 91894, 91902, 91903, 91917, 91918, 91920-91923, 91928, 91929, 91938-19941, 91943, 91945-91947, 93475); two were from Manus Province (LSUMZ 93465, 93466); three were from Northern Province (LSUMZ 93467, 93468, 93472); two were from Madang Province (LSUMZ 93473, 93474); two were from Milne Bay Province (LSUMZ 93469, 93470); one was from North Solomons Province (LSUMZ 93476). Lizards were collected 2001-2002, 2004-2006.

The left gonad was removed, dehydrated in ethanol, embedded in paraffin, sectioned at 5 μm and stained with Harris hematoxylin followed by eosin counterstain (Presnell and Schreibman, 1997). Enlarged follicles (> 3 mm diameter) and oviductal eggs were counted. Histology slides were deposited in LSUMZ. Male and female mean body sizes (SVL) were compared with an unpaired t test using Instat (vers. 3.0b, Graphpad Software, San Diego, CA).

Results

Male *L. smaragdina* were significantly larger than females (unpaired t test, $t = 2.4$, $df = 22$, $P = 0.026$). Two stages in the testicular cycle were noted: (1) spermiogenesis (sperm formation) in which the seminiferous tubules are lined by clusters of sperm or groups of metamorphosing spermatids; (2) regressed, in which the seminiferous tubules are reduced in size and contain mainly spermatogonia. Regressed testes were found in two juvenile males, LSUMZ 93467 (SVL = 75 mm) and LSUMZ 91928 (SVL = 77 mm). Males undergoing spermiogenesis were noted in the following months, samples sizes in parentheses: June (1), July (4), August (2), September

(1), October (2), November (1). These data along with April and May *L. smaragdina* males from Papua New Guinea undergoing spermiogenesis (Goldberg and Kraus, 2008) indicate a prolonged period of sperm formation. The smallest reproductively active *L. smaragdina* male measured 96 mm (LSUMZ 91903) and was from June.

Monthly stages in the ovarian cycle are in Table 1. Four stages were present; (1) quiescent, no yolk deposition; (2) early yolk deposition, basophilic vitellogenic granules are in the cytoplasm; (3) enlarged preovulatory follicles, > 3 mm diameter; (4) oviductal eggs. Mean clutch size for 10 females was 1.9 ± 0.32 , range = 1-2. The smallest reproductively active female (LSUMZ 93475) measured 83 mm SVL and was from September. One female from July (LSUMZ 91921) with oviductal eggs was undergoing concurrent yolk deposition for a subsequent clutch indicating *L. smaragdina* produces multiple clutches in the same year. This was also reported for a *L. smaragdina* female from Papua New Guinea from January (Goldberg and Kraus, 2008). The presence of *L. smaragdina* females with oviductal eggs from January and April from Papua New Guinea (Goldberg and Kraus, 2008) indicates a prolonged period of ovarian activity.

Table 1. Monthly stages in the ovarian cycle of 13 *Lamprolepis smaragdina* from Papua New Guinea. *female with oviductal eggs and concurrent yolk deposition for a subsequent clutch.

Month	<i>n</i>	Quiescent deposition	Early yolk > 5mm	Follicles >3 mm	Oviductal eggs
June	1	0	0	1	0
July	3	0	0	2	1*
August	6	2	0	3	1
September	2	1	0	1	0
September	1	0	0	1	0

Discussion.

Other studies on reproduction of *L. smaragdina* reported year-round egg production in the Philippines (Alcala, 1966; Auffenberg and Auffenberg, 1989). An identical mean clutch size (1.9 ± 0.32) was reported for *L. smaragdina* in the Philippines by Auffenberg and Auffenberg (1989). McCoy (2006) reported clutches of two eggs for *L. smaragdina* in the Solomon Islands. In view of the extensive range of *L. smaragdina*, (Uetz and Hallermann, 2010), examination of other populations are needed to determine whether geographic variation in the *L. smaragdina* reproductive cycle exists.

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Larval Periods of *Eurycea lucifuga* at Rock Face Surface Habitats in Southern Illinois

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Abstract.

The larval life history variation of the cave salamander, *Eurycea lucifuga*, was studied at two rock face surface sites in southern Illinois from January 1976 through January 1978. Hatchlings (those collected with yolk remnants and undifferentiated limbs) were 9-12 mm snout-vent length (SVL) and found from October 1977 through January 1978 at one site and November 1976 and November and December 1977 at the second site. Only two (out of a total of 352 larvae) metamorphic larvae (31 mm SVL) collected in April 1976 presumably overwintered a second year. Other large larvae reached only 26 mm SVL. Larval periods were estimated at seven to nine months long at one rock face site during both 1976 and 1977. Mean SVL's between the two rock face sites was significantly different.

Introduction.

Aspects of the larval life history of *Eurycea lucifuga* have been determined for cave habitats (Banta and McAtee, 1906; Myers, 1958; Rudolph, 1978; Petranka, 1998; Ringia and Lips, 2007; McDowell, 2008). The larval period at rock face (surface) habitats, though, has not been described. Characteristically associated with limestone cave and rock face pools and streams, larvae are difficult to find, making interpretations of larval life history data (hatchling sizes and seasons, metamorphic sizes, larval period length variation, and overwintering) more problematic than for other *Eurycea* spp. (Ireland, 1974; Semlitsch, 1980; Bruce, 1982a, b, 1985, 1986, 1988; Voss, 1993; Ryan, 1998; Freeman and Bruce, 2001). My purposes were to compare the larval life history and microgeographic variation of *Eurycea lucifuga* at two rock face sites in southern Illinois and with previously published data from a cave site (McDowell, 2008).

Variation in life history traits within amphibian populations at pond habitats may be seen as a response to unpredictable environments (Wilbur et al., 1974) and some species respond by increasing the variation or plasticity in their life history strategies (Beachy, 1993). Recently, Bruce (2005) discussed the larval life histories of stream dwelling plethodontids including *E. lucifuga* and believed that spelerpine plethodontids do not fit pond breeding biphasic amphibian models. He also noted that there is considerable inter- and intraspecific variation in salamander larval periods and metamorphic sizes. Environmental pressures (habitat size and stability, water temperatures, food bases and reproductive phenologies) of rock face surface and cave *E. lucifuga* populations probably differ and I wanted to measure and document aspects of the larval life history at rock face habitats and compare that to a cave population in southern Illinois.

Materials and Methods.

Two unnamed limestone rock face study sites approximately 2 km apart in Johnson Co. southern Illinois (designated as sites A and B) had small pools at their base which varied in size

Key Words: Cave salamander, *Eurycea lucifuga*, Larvae, Larval Life History

from 0.89 m in width and 0.07 m in depth at site A to 1.44 m in width and 0.24 m in depth at site B. These pools never completely dried but occasionally the surface froze during winter. Site A had a small stream 0.8 m in width and 0.1 m in depth leading from the pools. Most larvae were collected from beneath rocks with a few found in open water.

Larvae were sampled (with efforts to collect at least 20 individuals/month) every month from January 1976 through January 1978 at site A and August 1976 through December 1977 at site B. In some months larvae were not found although sampling efforts were made. At site A larvae were sampled from both the pools and stream. Sampling was not done in January 1977 at site A due to freezing. Larvae were sampled from two pools at site B. Larvae were collected with a small aquarium dip net and preserved in 10% formalin on the day of collection.

Snout-vent length (SVL) was measured from the tip of the snout to the anterior angle of the vent more than 30 d after preservation. Large larvae were inspected for metamorphic characters (reduction or absence of tail fins, gill remnants, juvenile coloration [light yellowish-red color] and circular black spots). Small larvae were inspected for yolk remnants and undifferentiated limbs to identify recent hatchlings and to determine the hatching period. There was no attempt to find deposited eggs. Collections of juveniles were made at site A in June 1976, sacrificed in 10% chlorotone, fixed and preserved in 10% formalin.

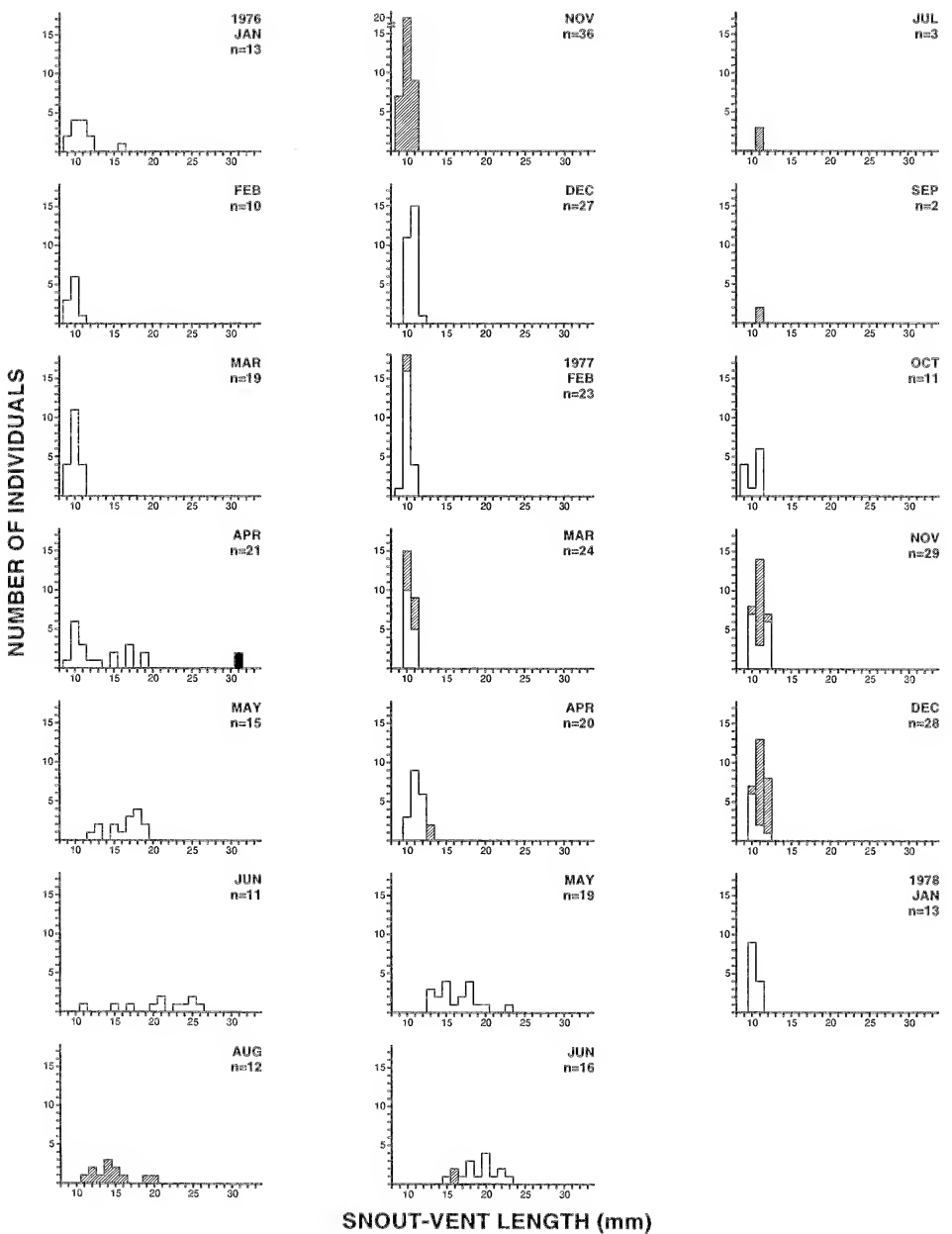
Sample sizes were 252 larvae and 5 juveniles from site A and 100 larvae from site B. Voucher specimens were deposited at Southern Illinois University at the Carbondale Vertebrate Fluid Collection. Larval SVL's are depicted as monthly size frequency histograms. Estimations of larval growth at site A determined from mean monthly larval SVL were described by linear regression equations. Mean sample size SVL of the two rock face sites was compared using a t test. Results are reported as mean \pm SEM with significance level at $\alpha = 0.05$.

Results.

In January 1976 small larvae (10.5 ± 0.48 mm SVL, $N=13$) without yolk remnants at site A (Fig. 1) were presumably from a 1975 fall or winter hatchling class cohort. One larger larva 16 mm SVL collected in January possibly suggested an early fall clutch. There was no significant change in the fall 1975 cohort SVL through the winter and to March 1976. Most estimated larval growth (10.72 ± 1.47 mm SVL, $N=47$) was from April through June with the greatest monthly growth (4.39 ± 0.63 mm SVL, $N=26$) from May through June. Estimated mean monthly growth rate of the 1975 year class cohort was 1.89 ± 1.76 mm and fit the regression line $Y = 2.03X + 6.68$, ($r^2 = 0.79$, $F = 15.39$, $P < 0.05$). Larvae reached a maximum mean SVL of 20.75 ± 1.35 mm SVL in June with the largest larva 26 mm SVL. All monthly size frequency distributions were unimodal except in April 1976 when two large metamorphic larvae 31 mm SVL were found with reduced tail fins, juvenile coloration and gill remnants. Larvae presumably metamorphosed during the summer as they were not found from July through November. Small larvae < 11 mm SVL from December 1976 (Fig. 1) were presumably from a late fall 1976 hatchling cohort.

In May 1977 larvae exhibited a wide size range and the greatest estimated monthly growth increase (5.20 ± 0.35 mm SV)L. They continued to grow rapidly from May through June (3.14 ± 0.27 mm SVL). The largest larvae were found in June with a mean SVL of 19.58 ± 0.57 mm SVL (maximum 23 mm SVL). Second year larvae were not found during the 1977 larval sampling period. Estimated mean monthly growth rate for the 1977 year class cohort at rock face site A was 1.78 ± 1.61 mm and fit the regression line $Y = 2.99X + 13.38$, ($r^2 = 0.76$, $F = 12.23$, $P < 0.05$). Most estimated growth (8.34 ± 0.46 mm SVL, $N=55$) occurred from April through June. Larvae presumably metamorphosed during the summer after a larval period of approximately 7 to 9 months based on a late fall hatching cohort and an absence of larvae from July through September.

FIG. 1. Distributions of snout-vent lengths of *E. lucifuga* larvae at sites A and B. Clear blocks represent larvae from site A, dark blocks represent metamorphic larvae from site A, and cross-hatched blocks represent larvae from site B. n represents number of individuals collected monthly.



At site A mean estimated monthly growth rates for the 1976 and 1977 year classes were similar but growth began during April in 1976 and May in 1977. Larvae from January through May 1977 had similar sizes as in 1976; a small SVL (9-12 mm) during winter with a larger SVL during early summer. Small juveniles (27-30 mm SVL, N=5) from June were only slightly larger than the largest first year larvae (23-26 mm SVL) collected in June 1976 and June 1977. From October 1977 through January 1978, I found recent hatchlings 9-12 mm SVL in length with yolk remnants and undifferentiated limbs, suggesting that hatching occurred from October 1977 through January 1978. Mean sample SVL at site A was 12.56 mm and with a large SEM of 16.69 which is interpreted as a wide SVL range present in the monthly samples. Undoubtedly though, yearly variation in oviposition and the hatching period probably occurred at site A.

In August 1976 at site B (Fig. 1) I found a wide size range (11-20 mm SVL). Larvae 9-11 mm SVL from November 1976 were recent hatchlings having yolk remnants and undifferentiated limbs. Small larvae 10-11 mm SVL were collected from February through September 1977. Larvae were not found in October, but newly hatched larvae 10-12 mm SVL with yolk remnants were collected in November and December 1977. The largest larva found at site B was 20 mm SVL. Metamorphosed individuals were not found and it could not be determined if any larvae underwent metamorphosis. Growth rates also could not be determined due to the very small monthly sample sizes. An extended ovipositional period is a possibility as small larvae 9-11 mm SVL were collected throughout the year. Mean monthly sample SVL was 11.22 ± 3.57 mm and with a small variance which is interpreted as very similar SVL's in the consecutive monthly samples and little variation in monthly growth rates.

Mean total sample size SVL for the rock face sites combined was $12.27 \pm .21$ mm and the two sites were significantly different when comparing mean SVL's ($t=3.137$).

Discussion.

Larval periods were different in some aspects when comparing the two cave salamander rock face populations. At site A only two larvae found in April (Fig. 1) possibly overwintered a second year while metamorphic larvae weren't collected at rock face site B. Smith (1961) found large overwintering larvae at rock face sites in LaRue Pine Hills Natural Research Area in southern Illinois but gave no actual sizes. The smallest metamorphic juveniles collected at site A (27-30 mm SVL) reflect the larger larval sizes (25-31 mm SVL) attained. Larvae were not found to overwinter (other than their birth year) more than one year at site B. At no time were metamorphs (juveniles or adults) found at site B, although larvae were found throughout the year. Larvae reached a much larger size at site A (31 mm SVL) than at site B (20 mm SVL) but this may be due to sample bias with fewer larvae (N=100) collected at site B than at site A (N=252). Alternatively, low food resources at site B may have possibly caused larvae to not reach metamorphic size and also explain why juvenile metamorphs were not found. It is probable that a low detection probability for juveniles occurred at site A as only five juveniles were found.

There were several larval life history differences when comparing the cave salamander populations from the rock face sites with the Union Co. cave site (McDowell, 2008). Larvae at the cave site overwintered 2-3 years and attained much larger sizes (to 36 mm SVL and with many individuals in that size range) than at the two rock face sites. Larvae at the cave population had a much longer hatching period (fall through spring). Hatchling size range (9-12 mm SVL) though, was similar for both rock face and cave sites.

Atkinson (1994) found that low temperatures slow the metabolism of some ectotherms, decreasing growth and developmental rates. Huppop (2000) found that caves have lower food resources than surface sites (prey bases may be different) and that cave organisms in general have

reduced metabolic and low growth rates. Gillieson (1996) determined that cave-dwelling species experience mild temperatures year round. The developmental rates of several amphibian species (*Ambystoma tigrinum*, *E. wilderae*, *Pseudotriton ruber*, and *Rana* spp.) were inversely proportional to temperature to a greater degree than growth, resulting in a larger size at metamorphosis at cold temperatures than at warm (Bruce, 1972, 1974, 1978; Bizer, 1978; Sexton and Bizer, 1978; Smith-Gill and Berven, 1979; Semlitsch, 1983). These factors could be the case for the cave population's longer larval life history. Bernadino and Reagan-Wallin (2002), though, found that species of three Plethodontid genera (*Desmognathus*, *Pseudotriton*, and *Eurycea*) of stream breeding salamanders had larger metamorphs at lower, warmer elevations and hypothesized this was due to habitat and phylogeny (Plethodontidae are lungless). Freeman and Bruce (2001) thought that the difference in larval period lengths of *E. guttolineata* was due to the degree of permanence of larval habitats.

The newly hatched larvae found in this study were larger (9-12 mm SVL) than those (8 mm SVL) reported by Myers (1958) but similar in size to those (10-13 mm SVL) found by Ringia and Lips (2007) at cave rimstone pools in Missouri. Newly hatched larvae at my study sites were found from October through May. Ringia and Lips (2007) found that yolk absorption averaged about 100 days.

The presence of small larvae, 9-12 mm SVL, over an extended time period is likely due to an extended reproductive season (Hutchinson 1956, 1958; Myers, 1958; Williams et al., 1985) and/or lengthy oviposition period (Hutchinson, 1966; Petranks, 1998; Phillips, et al., 1999; Ringia and Lips, 2007). Ringia and Lips (2007) believed the oviposition period to be five months lasting from August through December while my study shows the ovipositional period from October through January. An extended oviposition period may also cause some larvae to overwinter simply because they were not large enough when conditions favoring metamorphosis began to decline. Bruce (2005), though, suggests a maximization of time of metamorphosis (late spring or summer) rather than size for plethodontines. Eggs have been found in caves in late September in West Virginia (Green et al., 1966) and early January (Myers, 1958) and August through December (Ringia and Lips, 2007) in Missouri. Eggs have not been found at rockface habitats. Rockface seep and cave *E. lucifuga* populations may have different reproductive phenologies. Williams et al. (1985) found that the mating period in southern Illinois was July through September when the vasa deferentia reached maximum thickness and were packed with sperm, which correlates well with the October through January hatching period seen in this study. These factors may account for the variation in hatching and larval life histories seen in these Illinois populations. The rock face surface and cave populations (McDowell, 2008) of larval cave salamanders seemingly differed greatly (although numbers of cave site larvae are much fewer) in their responses (hatching periods, larval period lengths, overwintering and metamorphic sizes) to different environmental habitats. There is a need for additional studies of larval salamanders in differing habitat types with measures of environmental variables that may explain larval life history differences.

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Substrate and cover object choice by the Red-Backed Salamander (*Plethodon cinereus*)

Deciduous and coniferous forests differ in several characteristics such as microclimate (Cunnington et al. 2008), soil (Quideau et al. 1996; Scholes and Nowicki 1998), light (Nilsen 1985), and leaf litter (DeGraaf and Rudis 1990; Waldick et al. 1999). Such characteristics have the potential to influence the distribution and abundance of Red-backed Salamanders, *Plethodon cinereus* (e.g., DeGraaf and Yamasaki 2002; deMaynadier and Hunter 1998; Harper and Guynn 1999; Sugalski and Claussen 1997). Indeed, Red-backed Salamanders are frequently more common in forests made up of deciduous trees than forests with coniferous trees or pine plantations (e.g., DeGraaf and Rudis 1990; Harper and Guynn 1999; Pough et al. 1987; Waldick et al. 1999), but this does not always appear to be the case (e.g., Mathewson 2009). It may be that the observed distributions and relative abundances of Red-backed Salamanders in deciduous and coniferous forests may be the result of differences in population dynamics between habitats or behavioral responses to the habitats or both. Little is known about the behavioral responses of *P. cinereus* to deciduous and coniferous habitats. We conducted a field mesocosm experiment to examine whether *P. cinereus* behaviorally respond to deciduous and coniferous leaf litter habitats. We predicted that the salamanders would avoid the coniferous habitat and prefer the deciduous habitat. We also examined cover object and substrate choice.

Experimental arenas were constructed using plastic tubs (86 cm x 56 cm x 21 cm), each with soil and leaf litter collected from deciduous and coniferous forests found in the Denison University Biological Reserve (DUBR), Granville, Ohio. Each tub was divided in half with the soil and leaf litter of one habitat placed on one half, and the other habitat on the other half. Two cover objects, one made of wood (14.5 cm x 14.5 cm x 2 cm) and another of rock (concrete paver: 14.5 cm x 14.5 cm x 4 cm), were positioned in the middle of each half. Half of the arenas were placed under a deciduous canopy, while half were placed under a coniferous canopy. Salamanders were collected from under cover objects from throughout the DUBR and for each trial a single salamander was placed in the middle of the arena on the border of the substrates. After 60 minutes, we recorded their location in the arena (i.e., under the wood cover object, under the rock cover object, hidden in the leaf litter, or on the surface; and deciduous or coniferous habitat). Trials occurred during the afternoon to evening hours during late April and early May 2006. No salamander was used in > 1 trial. We used chi-square tests to compare salamander choices among habitat types and among microhabitat types.

The salamanders sampled did not prefer either substrate when canopies were pooled (Table 1; $\chi^2_1 = 1.58$, $P = 0.21$). When each canopy treatment was examined individually, we again found no significant preferences (Table 1; Deciduous canopy: $\chi^2_1 = 0.25$, $P = 0.62$; Coniferous canopy: $\chi^2_1 = 1.67$, $P = 0.20$). Salamanders showed clear preferences for particular cover objects, with more salamanders being found under leaf litter or the wood cover object, and only a very few being found under the rock cover object or on the surface (in leaf litter: 17, under wood cover object: 12, under rock cover object: 1, on surface: 1; $\chi^2_3 = 25.13$, $P = < 0.0001$).

In our experiment, Red-backed Salamanders showed no preference or avoidance for either deciduous or coniferous leaf litter, although there was a slight tendency to use the deciduous leaf litter more. These results do not support our hypothesis that the salamanders would avoid the coniferous litter. Our results suggest that the distribution and abundance of Red-backed Salaman-

ders in deciduous and coniferous forests may not be the results of behavioral responses. However, it may be that the results reflect the timing of our observations. Due to logistic constraints, we conducted our observations during the day and did not allow the salamanders to spend the night in the experimental arenas. Since Red-backed Salamanders are primarily active on the surface at night (Petranka 1998), their primary behavioral response might have been to find cover, regardless of the habitat type, as is suggested by the high proportion of salamanders found under the wood cover object and in the leaf litter.

Table 1. Choice of deciduous or coniferous habitats by *Plethodon cinereus* in the experimental arenas.

	Deciduous	Coniferous
Pooled	19	12
Under Coniferous Canopy	10	5
Under Deciduous Canopy	9	7

Our results also showed that Red-backed Salamanders strongly preferred the wood cover objects to the rock cover objects. Our results contrast with the observations made by Richmond and Trombulak (2009) that more Red-backed Salamanders are found under rocks than woody cover objects. However, our results are consistent with the several studies that have found a positive relationship between coarse woody debris and salamander abundance (e.g., Hicks and Pearson, 2003; Morneau et al., 2004; Waldick et al., 1999; Young and Yahner, 2003).

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Evaluation of the rate of artificial coverboard use by the salamander, *Plethodon cinereus*, in the vicinity of natural cover objects

Artificial cover objects (ACOs) can be effective tools for assessing the relative abundance of salamanders, and for monitoring salamander populations (Davis 1997; Fellers and Drost 1994; Houze and Chandler 2002), particularly Red-backed Salamanders, *Plethodon cinereus* (Carfioli et al. 2000; Grover 2006). In several studies, the number of salamanders, including Red-backed Salamanders, or amphibians has been shown to increase with increases in natural cover objects (NCOs) such as coarse woody debris (CWD) (e.g., Grover 1998; Hicks and Pearson 2003; Morneault et al. 2004; Young and Yahner 2003), although this relationship can vary depending on the quality of the CWD (e.g., McKenny et al. 2006). In addition, ACOs have been shown to allow *P. cinereus* to exist in areas, such as meadows and pastures, that lack NCOs (Riedel et al. 2008). In some populations, relatively few NCOs are occupied by Red-backed Salamanders (Richmond and Trombulak 2009). Thus one might expect that the efficacy of ACOs may be influenced by the proximity of ACOs to NCOs. We investigated if the number of Red-backed Salamanders found under an ACO is influenced by the number of natural cover objects (NCOs) located near the ACO. In addition, we examined whether the size of salamanders differs between ACOs and NCOs.

This study was conducted in the Denison University Biological Reserve in Granville, Licking County, Ohio on three sampling dates in late March and early April 2008. We used coverboards (ACOs) measuring 61 by 30 cm by 5 cm located in a deciduous woods. Coverboards had been in place for six years prior to this study. We measured the dimensions of the three closest natural cover objects (NCOs), including both logs and rocks, and measured their distance from each respective coverboard. We counted and then measured the SVL (to nearest mm) of all salamanders found under both ACOs and NCOs. For analyses we used the mean number or SVL of salamanders found under each cover object across all three sampling dates. For some analyses, we also corrected for the size of the cover objects by dividing the number of salamanders found under a cover object by the area of the cover object. We used paired t-tests to compare numbers of salamanders and SVL between ACOs and NCOs. We also used linear regression to examine the relationships between the number of salamanders under ACOs and the mean distance to the nearest NCOs, between the mean number of salamanders beneath ACOs and NCOs, and between the mean size of salamanders under ACOs and NCOs. Pairs of NCOs and ACOs with no salamanders were not included in the analyses.

Significantly more salamanders were found beneath ACOs (0.66 ± 0.096) than NCOs (0.29 ± 0.096) ($t_{30} = 3.80$, $P = 0.0007$). When the number of salamanders found per cover object was corrected for the area of the cover objects, there was no significant difference in the number of salamanders under each cover object type (ACO: 0.0004 ± 0.0002 ; NCO: 0.0006 ± 0.0002 ; $t_{30} = 1.57$, $P = 0.13$). Salamander size did not differ between ACOs (3.30 ± 0.26 cm) and NCOs (3.17 ± 0.26 cm) ($t_{10} = 0.49$, $P = 0.63$).

The number of salamanders per coverboard was positively related to the distance to the nearest NCOs (mean number of salamanders under ACO = $0.28 + 0.005$ mean distance to NCOs; $N = 37$, $r^2 = 0.15$, $P = 0.017$). The number of salamanders under ACOs was positively related to the number of salamanders under nearby NCOs (mean number of salamanders under ACOs = $0.44 + 0.42$ mean number of salamanders under NCOs; $N = 38$, $r^2 = 0.13$, $P = 0.026$). There was no relationship between the mean size of salamanders under an ACO and the mean distance to the

nearest NCOs ($N = 26$, $r^2 < 0.001$, $P = 0.99$). There was no relationship between the mean size of the salamanders under ACOs and the mean size of the salamanders under nearby NCOs ($N = 11$, $r^2 = 0.08$, $P = 0.39$).

Taken together, our results suggest that ACOs are effective at sampling Red-backed Salamanders, with more salamanders tending to be found under ACOs than NCOs, although this is in large part due to the larger area of the ACOs than the NCOs. Moore (2005) also found that the number of Red-backed Salamanders was related to the area of the cover object. In addition, we found that ACOs were used by more salamanders when the distance to the nearest NCO was greater, suggesting that ACOs may be more useful at detecting salamander presence or absence in areas with fewer or less dense NCOs. However, we also found a significant relationship between the number of salamanders under ACOs and nearby NCOs, suggesting that estimates of the relative abundance of the salamanders based on ACO and NCO surveys are likely to provide qualitatively similar results.

We also found that the size of *P. cinereus* found under ACOs and NCOs did not differ. This is similar to the results of previous studies (Houze and Chandler 2002; Marsh and Goicochea 2003; Monti et al. 2001; Richmond and Trombulak 2009). However, there may be concerns when juveniles are considered (e.g., Marsh and Goicochea 2003). These results suggest that ACOs likely provide a reasonable estimate of population size structure, at least for adult salamanders.

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Fall Mating Observation in the Eastern Wormsnake, *Carphophis amoenus amoenus*, in Maryland

White and White (2002) state "Mating behavior of the Eastern Wormsnake has not been observed in the field but there is evidence that it takes place in the spring and possibly also the fall." Fall mating and courtship in *Carphophis a. amoenus* have been reported in the southeastern United States by Russell and Hanlin (1999) and Wilson and Doreus (2004). It has also been reported in *Carphophis vermis* by Clark (1970). Females deposit 2 to 6 eggs under rotten logs, rocks, rotting vegetation etc. Mitchell (1994) states that the eggs hatch in late summer.

On 6 September 2008, at 10:25 PM (EDT), while driving the roads looking for amphibians and reptiles I stopped to examine an unusual Pickeral Frog, *Lithobates palustris*, 0.3 mi W of Severn Run on Dicus Mill Road. The frog was on the Northern side of the road near the edge where the leaf litter began. Out of the corner of my eye, I saw two Eastern Wormsnakes, *Carphophis a. amoenus*, in courtship. I watched as the male placed his head on the female's neck and moved his body repeatedly over hers trying to line up his hemipenes with her cloaca. In the excitement I disturbed them and they immediately disappeared under the leaf litter. I had wanted to collect them to verify the sexes, although I really had no doubt.

The temperature was 71 degrees, the humidity 83%, Dew Point 66, Barometric Pressure 29.80 inches and steady, the winds were Westerly at 4 mph, the sky was Partly Cloudy and the visibility was 10 miles. There was 2.48 inches of rain reported in this area throughout the day (Reference: Excel, Herps.New 3712-3714, from weather.com/airport profile).

I would like to thank Jerry D. Hardy, Jr. for his comments on this note.

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**Predation of Eggs of the Jefferson Salamander,
Ambystoma jeffersonianum, by the leech,
Macrobdella decora.**

Cargo (1960) first reported on this phenomenon in the Spotted Salamander, *Ambystoma maculatum* from a small Coastal Plain pond in Calvert County, Maryland. Here, the junior author, found six individual egg clusters of *Ambystoma jeffersonianum* on night of 12 April 2008 near Big Hunting Creek, Thurmont, Frederick County Maryland. These masses were attached consecutively, forming a spiral around a thin twig, roughly six to seven inches below the water surface. The mass appeared bright green from the algae surrounding the outer envelope of the egg mass. The outer envelope was clear, and the individual eggs and larvae were clearly visible.

The junior author, mistaking the egg masses for *A. maculatum*, due to the green algae, removed twelve individual eggs from the outer edge of a mass and brought them to the lab to perform a rearing study on the larvae. Prior to the eggs hatching the next day, a leech, here tentatively identified as *Macrobdella decora*, was found on the eggs. The leech was observed with its head inside of the jelly of the egg, nibbling on the head of the *A. jeffersonianum* larvae. The leech measured 5.1 cm in length. Cargo (1960) only observed the leech feeding on the egg, whereas here, the leech was observed actually feeding on the larvae itself. Moore (1923) has reported on the predation of frog eggs by this species of leech.

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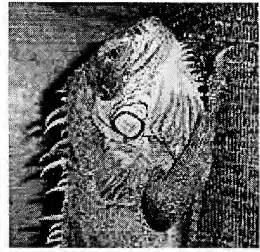
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The Editor

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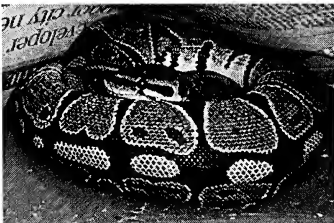
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